Review

Genetic variations of vitamin D receptor and vitamin D supplementation interaction in relation to serum vitamin D and metabolic traits: a systematic review and meta-analysis

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Abstract: *Background:* It is now becoming increasingly recognized that the effects of vitamin D supplementation may vary by several factors including vitamin D deficiency status, ethnicity, and/or the presence of genetic variants, which affect individual responses to supplementation. This study investigates the interaction between metabolic traits and circulating 25-hydroxyvitamin-D (25OHD) concentration with 4 polymorphisms of vitamin D receptor (VDR) including BsmI, ApaI, TaqI, FokI, and vitamin D supplementation. *Methods:* A systematic review and meta-analysis of papers until August 2021 on PubMed, The Cochrane Library, Scopus, Web of Science, Google Scholar, ProQuest, Science Direct, and Embase about the association between functionally relevant VDR variants and vitamin D supplementation on circulating 25OHD and metabolic traits. *Results:* A total of 2994 cases from 16 randomized controlled trial (RCT) studies were included in meta-analyses. There were no significant changes in the serum concentrations of 25OHD and metabolic traits after vitamin D supplementation in different variants of BsmI, ApaI, TaqI, and FokI polymorphism in the VDR gene in the overall analysis ($p>0.05$). However, the results showed there is significant interaction between these above VDR polymorphisms and vitamin D supplement on serum 25OHD level after subgroup analyses based on the study duration, gender, age, BMI, health status, Hardy–Weinberg Equilibrium, PCR, and race ($p<0.05$). *Conclusions:* The present meta-analysis demonstrates that the effect of vitamin D supplementation on serum 25OHD and metabolic traits is independent of genetic variants of the VDR gene (BsmI, ApaI, TaqI, and FokI). However, future trials should consider inter-individual differences and, in particular, should aim to clarify whether certain subgroups of individuals may benefit from vitamin D supplementation in the context of metabolic health.

Keywords: vitamin D supplementation, vitamin D receptor, metabolic, polymorphisms, meta-analysis

Introduction

One of the most serious public health issues in the world is vitamin D insufficiency, which has been associated with extra-skeletal disorders such as cardiovascular disease, hypertension, diabetes and insulin metabolism, metabolic syndrome, and dyslipidemia [1]. Therefore, there has been a significant increase in the consumption of vitamin D supplements in recent years [2].

The effect of vitamin D is modulated according to genotypes in known genes; in particular, the VDR gene, which plays a key role in regulating vitamin D metabolism [3]. Additionally, VDR is the main mediator for regulating the transcription of several genes involved in cellular growth, differentiation, apoptosis, angiogenesis, inflammation, and metastasis [4]. The Human VDR gene is found on chromosome 12q13.11 [5]. Four types of VDR polymorphisms consisting of FokI, BsmI, ApaI, and TaqI are associated with

serum levels of 25-OH vitamin D and metabolic traits with contradictory results, likely attributable to genetic variations [6]. BsmI (rs1544410 G>A), ApaI (rs7975232 C>A), and TaqI (rs731236 T>C) are found at the 3′ untranslated regions (3′ UTR) of the gene VDR, which not change the amino acid sequence of the translated protein, while can disrupt the reliability of mRNA thus impact on the efficacy of encoded protein [7, 8]. FokI (rs2228570 G>A) is found within the 5′ ends near the promoter region, which could produce a modified protein [9].

Several studies have shown the association of SNP of the VDR gene with serum 25-hydroxy vitamin D (25(OH) D) level and metabolic traits. Despite some evidence suggesting that vitamin D supplementation can improve metabolic factors and serum vitamin D status, this has not happened in several populations.

Therefore, we hypothesized that differences in responses to vitamin D supplementation may partially be explained by the genetic makeup and variations of VDR polymorphism. Importantly, polymorphisms in VDR genes related to vitamin D metabolism as potential contributors to these divergent results. Due to these above points, serum levels of 25 (OH) D may differ between individuals, even with a particular intake dose of vitamin D. Despite that to date, there is massive research on the roles of vitamin D supplementation or VDR separately on bone and metabolic diseases, while no meta-analysis and systematic review have been carried out to scrutinize the biological interaction of vitamin D supplementation and its receptor (VDR) on vitamin D regulated pathways in the context of serum vitamin D level and metabolic traits.

Methods

Protocol

The reported items for systematic reviews and meta-analysis (PRISMA) guidelines were applied for designing this study [10].

Search strategy

The first author (M.R) carried out the primary search and the second author (F.A) re-checked the obtained articles; the difference between the two authors was eliminated by a third author (A.R). A comprehensive search was conducted using the online databases containing PubMed, The Cochrane Library, Scopus, Web of Science, Google Scholar, ProQuest, Science Direct, and Embase up to August 2021. We used search keywords: ("vitamin D receptor gene" OR "VDR gene" [tiab]) AND "supplementation*[Title/Abstract"]) OR "vitamin D"[Mesh]) OR "25-OH vitamin D" [tiab])) AND "biochemical markers*[Title/Abstract]" OR "metabolic"[Mesh]) OR "serum vitamin D"[Mesh]) OR "vitamin D level"[Mesh]) OR "lipid profile"[Mesh]) AND ("intervention studies" OR "intervention" OR "controlled trial" OR "randomized" OR "randomized" OR "random" OR "randomly" OR "placebo" OR "assignment" OR "blind").

Eligibility criteria

Our search is restricted to studies published in randomized placebo-controlled trials (RCTs). Publication time and language were regarded without any restrictions. Evaluation of the interaction of VDR polymorphisms and vitamin D supplementation on metabolic traits and serum vitamin D; including available genotype frequencies of BsmI, ApaI, TaqI, and FokI; that reported sufficient data serum 25-OH vitamin D, BMI, lipid profile (LDL-C, HDL-C, TC, and TG), MDA and TNF-a values (mean and standard deviation) for different variants of VDR gene. Exclusion criteria were as follows: (1) incomplete data on SNPs of VDR gene, metabolic traits, and serum 25-OH vitamin D; (2) no available data on the distribution of VDR gene SNPs in groups of case and control; (3) abstracts, case reports, conference report, reviews, academic thesis, duplicate studies and book seasons.

Data extraction

Two independent investigators (R. Gh and M.R) assessed eligible articles and extracted the relevant information using End-Note software, version X7 (Thomson Reuters). Subsequently, extracted articles were categorized by author's first name, publication year, country of study, sample size, allele frequency, Study duration, gender, age, BMI, health status, HWE (Hardy–Weinberg Equilibrium), PCR type, race and dosage of vitamin D supplementation. Eventually, all human RCTs (either parallel or cross-over) that had assayed the efficacy of vitamin D Supplementation on serum 25-OH Vitamin D or metabolic traits according to SNPs of the VDR gene were included. The data for serum 25-OH vitamin D and metabolic traits were transformed into identical units and mean differences in measurements between subjects with different SNPs of the VDR gene were computed. Any possible different assessment was resolved by a third researcher (A. R). If data was indicated in different ways, standard formulas were carried out to obtain the same units. Additionally, articles with different gene variants of VDR of participants were considered separate articles.

Quality assessment

We assessed the quality of these included studies with the Cochrane Risk of Bias tool, containing: random sequence

generation, allocation concealment, blinding for researchers, participants, staff, and analyser, imperfect outcome data, selective reporting, and other sources of bias. Based on the Cochrane guideline handbook, the terms "yes", "no", and "unclear" are considered to as low, high, and unknown risk of bias, respectively. The quality of the study was scored as follows: low-quality (low risk for equivalent and less than three items), fair-quality (low risk more than three items), and high-quality (low risk for all items) [11]. Finally, two researchers (M. R and R.GH) conducted and completed quality assessments separately.

Statistical analysis

The meta-analysis was performed via STATA, version 14 (STATA crop, College Station, TX, USA). When there is no heterogeneity, we employed the fixed-effects model, otherwise, the random-effects model was used. The pooled weighted mean difference (WMD) and 95% confidence interval (CI) were used to assess the effects of vitamin D supplementation on serum level of 25-OH vitamin D and metabolic traits based on genetic models including dominant model, recessive model, and co-dominant model. Means and standard deviation (SD) of the measurements (25-OH vitamin D, LDL-C, HDL-C, TC, TG, MDA, and TNF-α) obtained for the intervention and placebo groups were used to assess the overall estimates with the same units. If the SD of the mean difference was not reported or indicated with a different unit, we computed and converted it via the standard formulas. We assessed the heterogeneity across studies via the Q-test and the I^2 index, these scores of $I^2 \geq 25\%$, $I^2 \geq 50\%$, and $I^2 \geq 75\%$ were considered as low, moderate, and high heterogeneity, respectively. If heterogeneity was moderate or high ($I^2 \geq 40\%$), we carried out subgroup and sensitivity analysis. Subgroup analysis was conducted based on the following characteristics: study duration (>12 weeks vs. ≤12 weeks), gender (Female or Both), age (≥55 vs. <55 years), BMI (≥30 vs. <30 kg/m^2), health status (healthy, unhealthy, vitamin D deficiency), HWE (Hardy–Weinberg Equilibrium) (Yes or NO), PCR type (RFLP or Other) and race (Asian or Non-Asian). Any potential bias was tested by Begg's test (funnel plot method) and Egger's linear regression test. A $p<0.05$ was considered a significant level [12].

Results

Study selection

The initial search recognized 357 articles. Of these, 78 articles were removed due to duplication. After title and abstract assessment, 29 relevant articles were included. Among these, 13 studies were excluded due to the following items: insufficient data (n=3) or unrelated polymorphism of the VDR gene (n=7) and another 6 had not required design (n=3). Finally, 16 articles were included in the present meta-analysis (Figure 1).

Characteristics of eligible articles

The selected studies have been published between 1994 and 2019. Additionally, all included studies were RCT studies with a total of 2994 subjects. Twelve studies [6, 9, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22] were conducted on Asian participants, studies in Europe [23, 24], and another two were performed in Brazil [25] and non-Hispanic white Americans [26]. Eleven studies designed a parallel-arm, while five investigations used a single-arm design. Among these, ten investigations were conducted only by women, while other studies were carried out on both genders. Moreover, the frequency of VDR polymorphism genotype and allele in both intervention and controls group as follow: (BsmI: BB+Bb=1077, bb=239; ApaI: AA+Aa=802, aa=135; FokI: FF+Ff=1171, ff=172; TaqI: TT+Tt=955, tt=150) were included in analysis. All studies followed the HWE. Detailed characteristics of the included trial were presented in Table 1.

Meta-analyses result for FoKI polymorphisms of VDR gene and vitamin D supplementation on serum 25-OH vitamin D

Seven investigations were eligible for evaluation of the interaction between FoKI polymorphisms in VDR and vitamin D supplementation on serum level of 25-OH vitamin D. FoKI polymorphism of the VDR gene in the recessive model (ff vs. Ff+FF) did not show any significant effect of vitamin D supplementation on 25-OH vitamin D (WMD: 3.22 ng/ml, 95% CI: −12.82 to 19.26, p=0.694) compared to the control group in different genotypes. Furthermore, we observed a significant heterogeneity for serum 25-OH vitamin D (I^2=97.8%, p=0.000) (electronic supplementary material [ESM] 1, Figure 1). Subgroup analyses were conducted based on the main sources of heterogeneity of VDR genes FoKI including the age (≥55 vs. <55 years), BMI (≥30 vs. <30 kg/m^2), duration time of supplementation (>12 and ≤12 weeks), health status (healthy, unhealthy, vitamin D deficiency), HWE (Hardy–Weinberg Equilibrium) (Yes or NO), PCR type (RFLP or Other) and gender (Female or Both).The results of subgroup analyses indicated positive significant for FokI, in age <55 year (WMD: 10.53 ng/ml, 95% CI: 7.72 to 13.34, p=0.006), BMI≥30 (WMD: 16.51 ng/ml, 95%

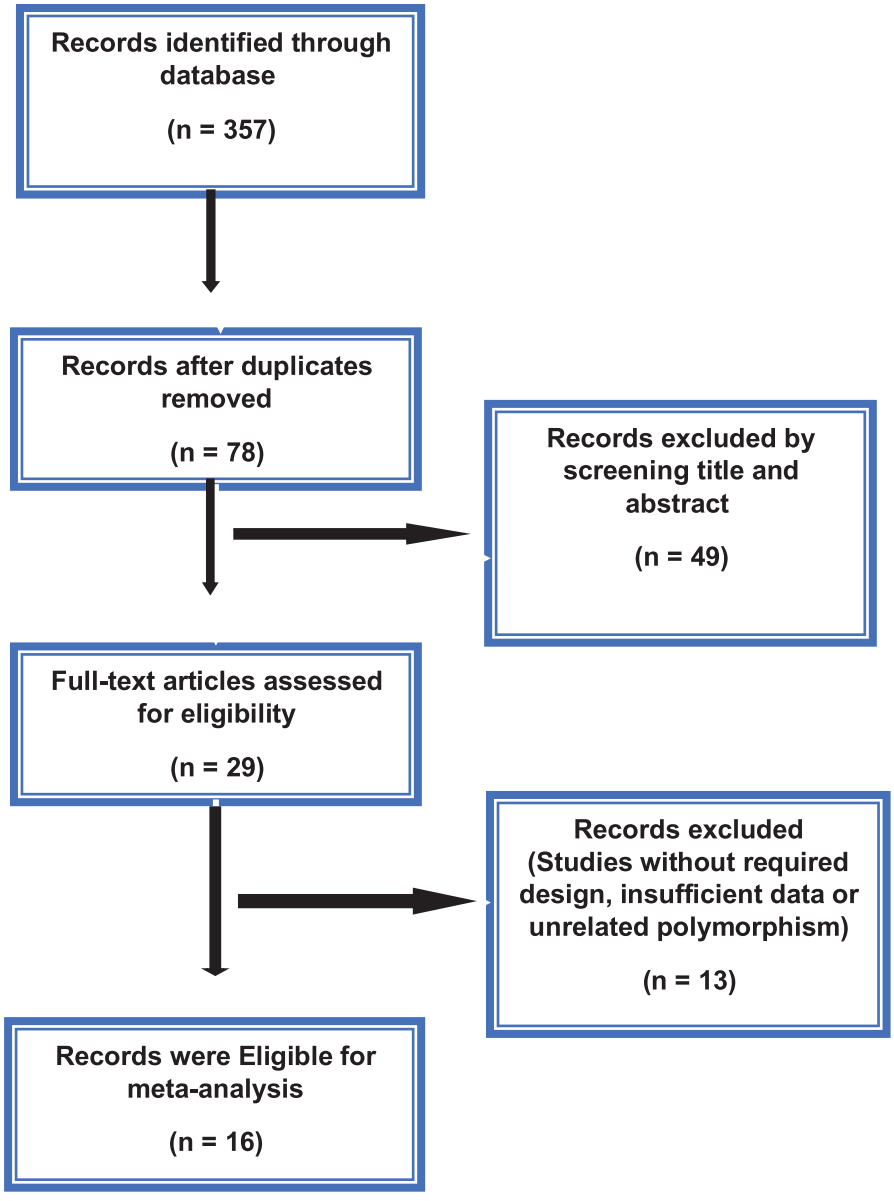


Figure 1. Preferred reporting items for systematic reviews and meta-analysis flow diagram of study selection process.

CI: 13.05 to 19.98, p=0.001), duration time ≤12 weeks (WMD: 25.4 ng/ml, 95% CI: 21.53 to 29.27, p=0.002), unhealthy status (WMD: 13.19 ng/ml, 95% CI: 10.62 to 15.76, p=0.007), HWE (N/A) (WMD: 14.65 ng/ml, 95% CI: 11.56 to 17.74, p=0.001), RFLP-PCR (WMD: 21.04 ng/ml, 95% CI: 17.67 to 24.42, p=0.000), females (WMD: 34.52 ng/ml, 95% CI: 30.19 to 38.88, p=0.001). Thus, in the above subgroups vitamin D supplementation significantly increase serum 25-OH vitamin D in the ff genotype compare with the FF+Ff genotype (Figure 2).

Meta-analyses result for FoKI polymorphisms of VDR gene and vitamin D supplementation on BMI, LDL-C, HDL-C, TC and TG

With the merge of pooled effect sizes, we found no significant effect of vitamin D supplementation in the recessive model (ff vs. Ff+FF) on BMI, LDL-C, HDL-C, TC and TG (ESM 1, Figure 2). Heterogeneity between investigations was not significant (p>0.05).

Table 1. Characteristics of the eligible studies

First Author/ Year/Country	SNPs	Study design/duration / Sample size	Evaluated Markers	Study population/Intervention type
1) Shab-bidar/ 2015/Iran	BsmI(rs1544410)	Parallel/12wBB (n=17) Bb (n=83) bb (n=40)	25OH, FBS, HbA1c, Weight, FM, BMI, WC, IL6, TNFa, hs-CRP, SAA, Endothelin, E-selectin, MMP, MDA, TAC, SOD, GSH, GSH-Px	140 T2DM subjects mean age >50 (500 ml/day) 1) VitD-fortified doogh (170 mg calcium+500 IU vitD)/250 ml 2) Plain doogh, (170 mg calcium, no vitamin D/250 ml)
2) Cavalcante/2014/ Brazil	BsmI(rs1544410)	Double-blind/Parallel/4w BB/Bb (n=12) bb (n=7)	25OH, PTH, Ca, Us-CRP, AGPA, MDA, TAC	40 elderly women age ≥60 with vitD insufficiency (52.5–72.5) nmol/l 1) Four vitD capsules (50,000 IU) 200,000 IU cholecalciferol/day. 2) Placebo capsules
3) Graafmans/1994/ Amsterdam	BsmI(rs1544410)	Parallel/1, 2 years BB (n=13) Bb (n=22) bb (n=11)	25OH, 1,25(OH)2D PTH, BMD femoral neck Osteocalcin	81 women, age >70, 1) Vitamin D (400 IU/daily) 2) Placebo tablets
4) Mohseni/2015/ Iran	BsmI(rs1544410) APaI(rs7975232) TaqI(rs731236) FokI(rs2228570)	Double-blind/Parallel/8w BB (n=7) Bb (n=11) bb (n=8) AA (n=11) Aa (n=11) aa (n=4) TT (n=8) Tt (n=15) tt (n=3) FF (n=3) Ff (n=19) ff (n=4)	TNF-α, TGF-β, TAC	56 breast cancer women age <50 1) Vitamin D 50,000 IU/week 2) Placebo capsules
5) Neyestani/ 2012/Iran	FokI(rs2228570)	Parallel/12wFF (n=55) Ff (n=47) ff (n=35)	25OH, BMI, WC, SBP DBP, FBS, Ins, Hb1c, TC, TG, LDL, HDL, iPTH, il-2, hs.CRP, SAA Endothelin, E-selectin MMP-9, ACR, IL4, IL6, IL10, TNFa, IF NY	140 T2DM subjects age >50 (500 ml/day) 1) VitD-fortified doogh (170 mg calcium+500 IU vitD) /250 ml 2) Plain doogh, (170 mg calcium, no vitamin D/250 ml)
6) Arabi/2009/Iran	BsmI(rs1544410) TaqI(rs731236) APaI(rs7975232)	Double-blind/Parallel/48w BB (n=31) Bb (n=88) bb (n=48) TT (n=54) Tt (n=89) tt (n=24) Not reported	25OH, (Total hip, Femoral neck, Forearm)BMC (Total body, Spine, Total hip, Femoral neck, Forearm Total body)BMD (Spine, Total hip Femoral neck Forearm Total body) area Height Lean mass	167 girls aged 10–17 years 1) Vitamin D3 preparation oil 1400 IU weekly (low dose group), or 14,000 IU weekly (high dose group) 2) Placebo oil
7) Sanwalka/ 2015/India	FokI(rs2228570)	Single-Arm Non-Randomized/ 12m FF (n=48) Ff (n=44) ff (n=10)	25OH, PTH, Ionized Ca, BMC, BA, BMD Lean body mass	32 girls aged 10–17 years 1) 30,000 IU vitamin D3/3m and 500 mg of elemental calcium/daily
8) Al-Daghri/2017/ Saudi Arabia	BsmI(rs1544410) APaI(rs7975232) TaqI(rs731236) FokI(rs2228570)	Single-Arm Non-Randomized/ 12m BB (n=55) Bb (n=103) bb (n=41) AA (n=87) Aa (n=84) aa (n=28) TT (n=54) Tt (n=99) tt (n=46) FF (n=113) Ff (n=70) ff (n=16)	25OH, BMI, SBP, DBP, FBS, Ins, Hb1-c, TC, TG, LDL, HDL	204 T2DM patient baseline 25OH <50 1) Received 2000 IU/daily vitamin D3 2) Did not have placebo/control group
9) Kazemian/ 2019/Iran	BsmI(rs1544410) APaI(rs7975232) TaqI(rs731236) FokI(rs2228570)	Single-Arm Non-Randomized/ 12w BB (n=82) Bb (n=74) bb (n=20) AA (n=83) Aav (n=68) aa (n=25) TT (n=70) Tt (n=89) tt (n=17) FF (n=82) Ff (n=72) ff (n=22)	IFNB, s-ICAM-1s-VCAM-1, IL6TNFa, PAI-1hs-CRP, MMP9E-cadherin	147 overweight and obese women 25–65 years' subjects, breast cancer 1) 4000 IU/daily vitamin D3 daily

Table 1. Characteristics of the eligible studies (Continued)

First Author/ Year/Country	SNPs	Study design/duration / Sample size	Evaluated Markers	Study population/Intervention type
10) Kazemian/2019/ Iran	BsmI(rs1544410) APaI(rs7975232) TaqI(rs731236) FokI(rs2228570)	Single-Arm Non-Randomized/ 12w BB (n=66) Bb (n=65) bb (n=16) AA (n=68) Aa (n=52) aa (n=18) TT (n=57) Tt (n=67) tt (n=14) FF (n=64) Ff (n=66) ff (n=17)	BMI, WC, HCFat mass, Muscle mass, Trunk fata Visceral fat, LDL, HDL, TC, TG	147 overweight and obese women 25–65 years' subjects, breast cancer 1) 4000 IU/daily vitamin D3 daily
11) (22)/Iran	BsmI(rs1544410) APaI(rs7975232) TaqI(rs731236)FokI (rs2228570)	Single-Arm Non-Randomized/ 12w BB (n=82) Bb (n=74) bb (n=20) AA (n=83) Aa (n=68) aa (n=25) TT (n=70) Tt (n=89) tt (n=17) FF (n=82) Ff (n=72) ff (n=22)		
12) Pérez-Alonso/ 2012/Spain	BsmI(rs1544410) APaI(rs7975232) TaqI(rs731236) FokI (rs2228570	Parallel/12w BB (n=82) Bb (n=74) bb (n=20) AA (n=83) Aa (n=68) aa (n=25) TT (n=70) Tt (n=89) tt (n=17)	25OH, Bc12, 8-OhdgMDA, TACSOD	176 overweight and obese women 25–65 years' subjects, breast cancer 1) 4000 IU/daily vitamin D3 daily
13) Mohseni/ 2015/Iran	BsmI(rs1544410) APaI(rs7975232) TaqI(rs731236) FokI(rs2228570)	Double-blind/Parallel/8w BB (n=2) Bb (n=16) bb (n=4) AA (n=9) Aa (n=9) aa (n=5) TT (n=4) Tt (n=13) tt (n=6) TT (n=4) Tt (n=13) tt (n=6) FF (n=7) Ff (n=19) ff (n=2)	25OH	56 breast cancer age <50 1) Vitamin D 50,000 IU/week 2) Placebo capsules
14) Barry/2014/US (non-Hispanic whites)	APaI(rs7975232) TaqI(rs731236)	Double-blind/Parallel/1year AA (n=9) Aa (n=9) aa (n=5) TT (n=4) Tt (n=13) tt (n=6)	25OH	897 non-Hispanic whites 45–75 years old (good general health) 1) VITAMIN D3 (1000 IU/d) 2) Calcium carbonate (1200 mg/d elemental calcium) 3) Vitamin D plus Calcium4) Placebo
15) Pang Yao/2013/ Chinese	FokI(rs2228570)	Double-blind/Parallel/20w FF (n=82) Ff (n=72) ff (n=22)	25OH	448 Chinese with vitamin D deficiencyx 1) 2000 IU/d vitamin D3 a 2) Placebo
16) Hu/2017/ Chinese	FokI(rs2228570) (21)(rs1544410)	RCT/30m FF (n=82) Ff (n=72) ff (n=22) BB (n=2) Bb (n=16) bb (n=4)	25OH	112 T2DM 50 years and older average serum 25(OH)D level was 22.71±6.87 ng/m 1) 800 IU/d vitamin D3

Meta-analyses result for BsmI polymorphisms of VDR gene and vitamin D supplementation on serum 25-OH vitamin D

Eight studies were included for evaluation of interaction between BsmI SNP of VDR gene and vitamin D supplementation on serum level of 25-OH vitamin D. There is not any significant effect of vitamin D supplementation on 25-OH vitamin D in BsmI SNP under recessive model (BB, Bb) vs bb) compared to the control group in different genotypes (WMD: 1.99 ng/ml, 95% CI: −2.55 to 6.53, p=0.390). There was high heterogeneity between studies (I^2=89.6%, p<0.001) (ESM 1, Figure 3). The chief sources of heterogeneity for BsmI SNP of VDR gene comprising of age (≥ 55 vs. <55 years), BMI (≥ 30 vs. <30 kg/m^2), duration time of supplementation (>12 and ≤12 weeks), health status (healthy, unhealthy, vitamin D deficiency), HWE (Hardy–Weinberg Equilibrium) (Yes or NO), PCR

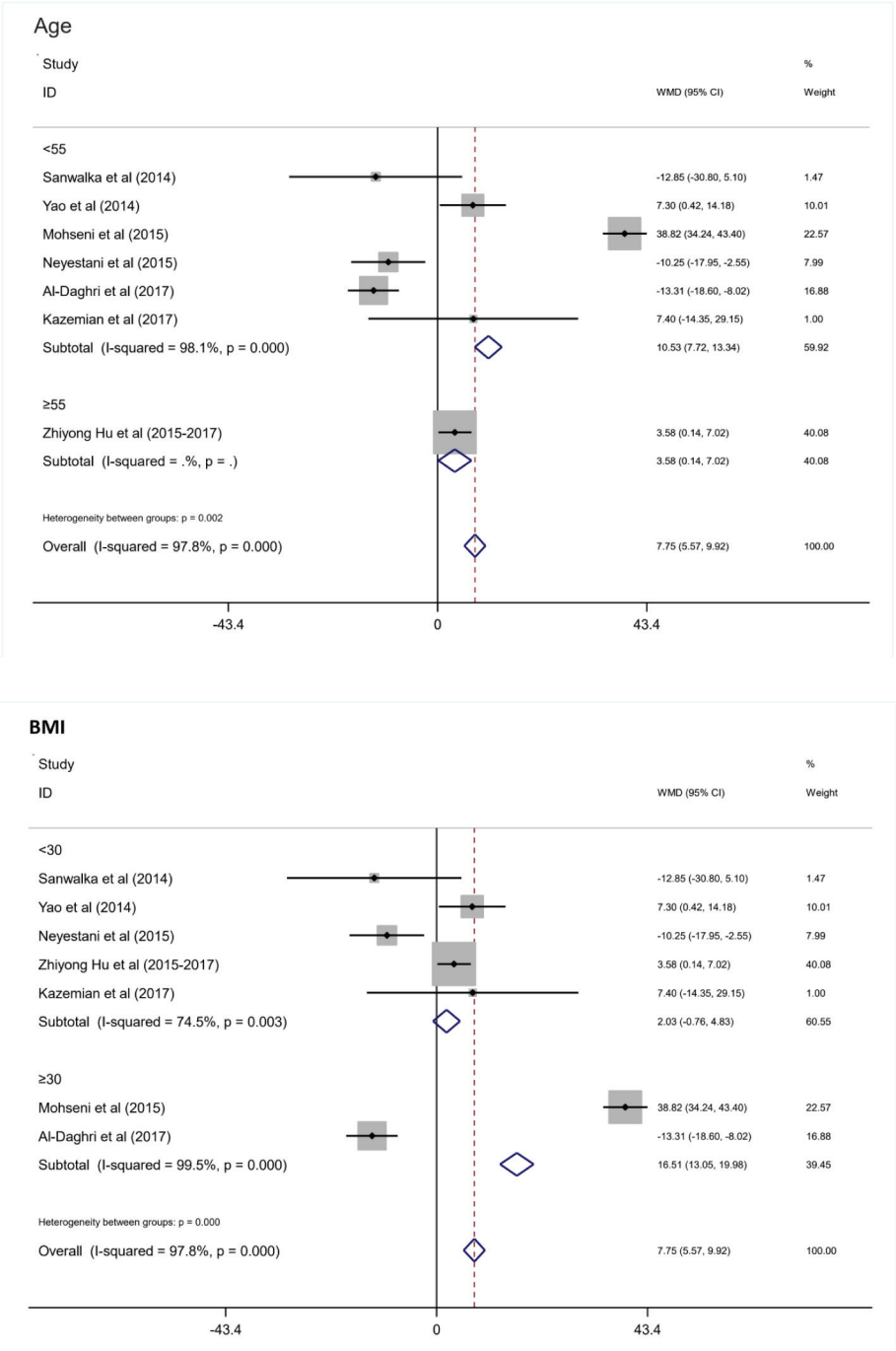


Figure 2. Subgroup analyses of vitamin D supplementation on serum concentrations of 25-OH vitamin D among FoKI ff vs (FF.Ff) polymorphism.

type (RFLP or Other), gender (Female or Both) and race (Asian or non-Asian). Pooled results from the subgrouping have shown that serum 25-OH vitamin D level significantly modify after vitamin D supplementation in recessive model (BB, Bb vs bb) only in age ≥55 year (WMD: 7.59 ng/ml, 95% CI: 6.22 to 8.96, p<0.001), BMI≥30 (WMD: 5.49 ng/ml, 95% CI: 4.26 to 6.73, p<0.001), duration time ≤12 weeks (WMD: 6.28 ng/ml, 95% CI: 5.04 to 7.53, p<0.001), healthy (WMD: 7.05 ng/ml, 95% CI: 5.63 to 8.48, p<0.001) and vitamin D deficiency status (WMD: 2.52 ng/ml, 95% CI: 0.90 to 6.14, p<0.001), HWE (Yes and No) (WMD: 5.98 ng/ml, 95% CI: 4.62 to 7.33, p<0.001 and WMD: 3.22 ng/ml, 95% CI: 1.01 to 5.42, p<0.001, respectively), Other-PCR (WMD:

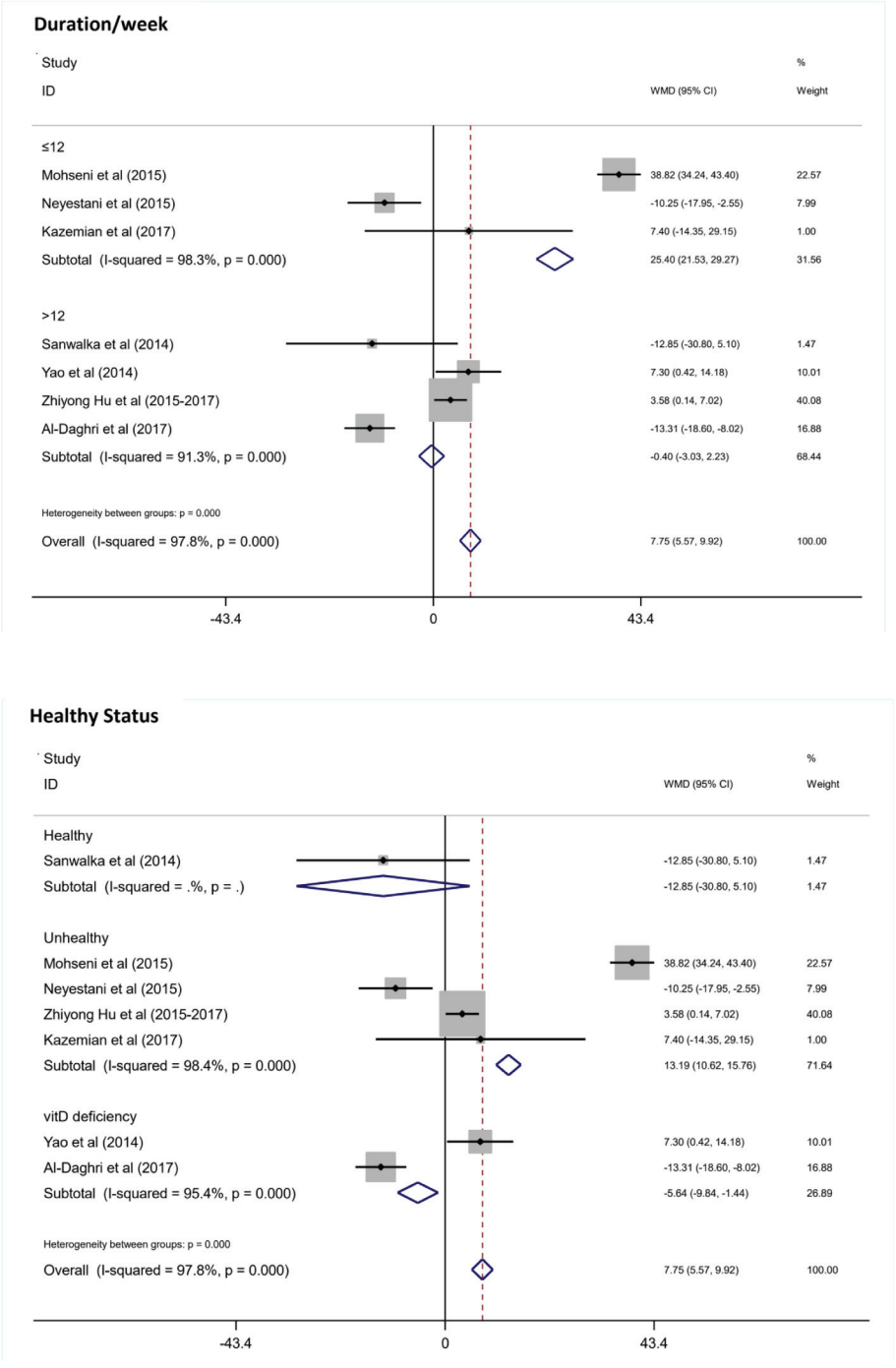


Figure 2. Continued.

8.26 ng/ml, 95% CI: 6.80 to 9.73, p<0.001), Non-Asian (WMD: 7.59 ng/ml, 95% CI: 6.22 to 8.96, p<0.001) and females (WMD: 6.16 ng/ml, 95% CI: 5.02 to 7.50, p<0.001) (Figure 3). According above subgroup analysis, vitamin D supplementation significantly increase serum 25-OH vitamin D in bb genotype compare with BB, Bb genotype.

Meta-analyses result for BsmI polymorphisms of VDR gene and vitamin D supplementation on BMI, MDA and TNF-α

The overall results showed that vitamin D supplementation did not have any significant effect on BMI, serum concentration of MDA, and TNF-α compared to the control group

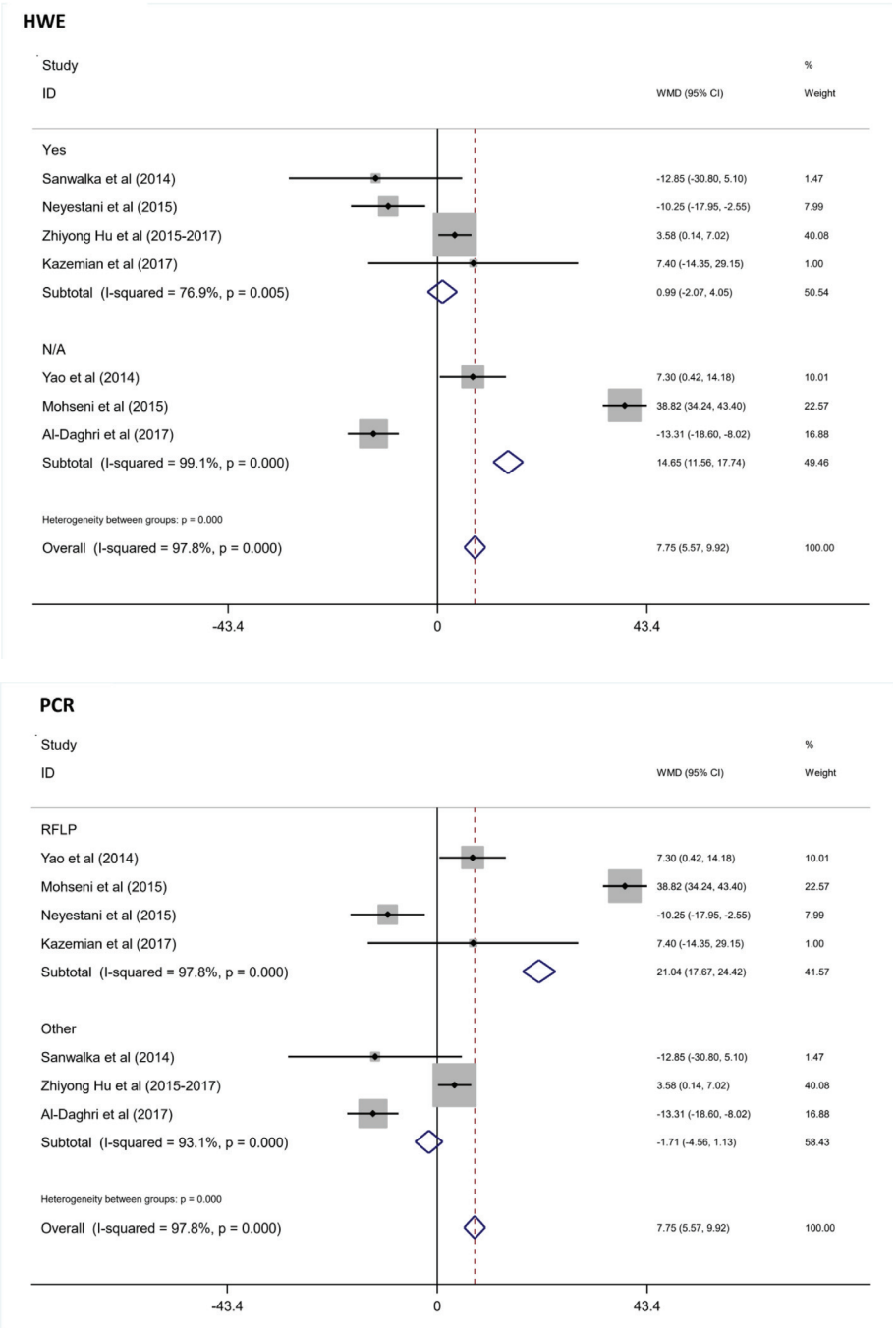


Figure 2. Continued.

in the recessive model (bb vs. Bb+BB) (ESM 1, Figure 4, p>0.05).

Meta-analyses result for TaqI polymorphisms of VDR gene and vitamin D supplementation on serum 25-OH vitamin D

The pooled estimation from 6 studies shown that vitamin D supplementation did not significant effect on 25-OH vitamin D in different TaqI polymorphism (TT, Tt vs tt) (WMD: −1.97 ng/ml, 95% CI: −6.03 to 2.08, p=0.741). Furthermore, heterogeneity between investigation was considerable (I^2=88.8%, p<0.001). (ESM 1, Figure 5). Subgroup analysis expressed that vitamin D supplementation decrease serum 25-OH vitamin D in younger and obese subjects (< 54 years (WMD: −7.30 ng/ml, 95% CI: −9.52 to −6.08, p<0.001) and BMI≥30 kg/m^2 (WMD: −8.37 ng/ml, 95% CI: −10.64 to −6.09, p<0.001), less and equal than 12 weeks

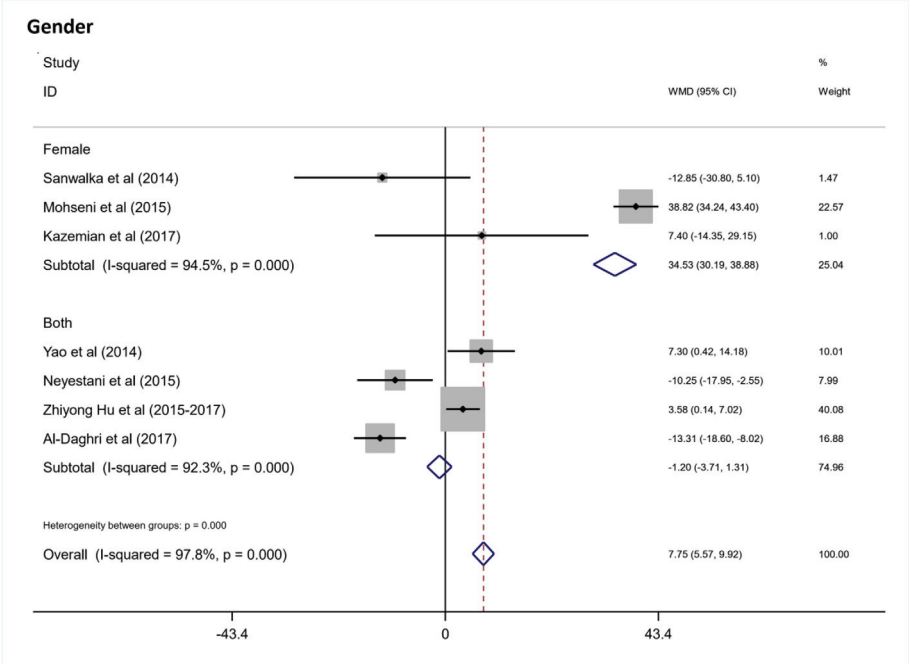


Figure 2. Continued.

for duration (WMD: −5.19 ng/ml, 95% CI: −6.69 to −3.69, p<0.001), unhealthy status (WMD: −9.48 ng/ml, 95% CI: −11.99 to −6.98, p<0.001), HWE (N/A) (WMD: −8.37 ng/ml, 95% CI: −10.64 to −6.09, p<0.001), RFLP-PCR (WMD: −8.46 ng/ml, 95% CI: −10.9 to −6.02, v0.001) and Asian (WMD: −7.30 ng/ml, 95% CI: −9.52 to −5.01, p<0.001), while increase concentration of serum 25-OH vitamin D in women under recessive model of TaqI polymorphism in tt genotype compare with TT, Tt genotype (WMD: 7.46 ng/ml, 95% CI: 6.22 to 8.70, p<0.001) (Figure 4).

Meta-analyses result for ApaI polymorphisms of VDR gene and vitamin D supplementation on serum 25-OH vitamin D

The pooled effect size suggested that the serum level of 25-OH vitamin D was not significant change after supplement therapy with vitamin D in variant genotypes of ApaI polymorphism in recessive model (AA, Aa vs aa) (WMD: 4.42 ng/ml, 95% CI: −4.48 to 13.32, p=0.350). There was high heterogeneity between studies (I^2=96.2%, p<0.001) (ESM 1, Figure 6). Subgroup analysis shown that vitamin D supplementation did significant effect on 25-OH vitamin D level in subjects with age <55 year (WMD: 20.6 ng/ml, 95% CI: 17.5 to 23.7, p<0.001), BMI≥30 (WMD: 21.74 ng/ml, 95% CI: 18.59 to 24.89, p<0.001), duration time ≤12 weeks (WMD: 4.07 ng/ml, 95% CI: 2.66 to 5.49, p<0.001), unhealthy status (WMD: 25.98 ng/ml, 95% CI: 22.51 to 29.45, v0.001), HWE (N/A) (WMD: 21.74 ng/ml, 95% CI: 18.59 to 24.89, p<0.001), RFLP-PCR (WMD: 25.98 ng/ml, 95% CI: 22.51 to 29.45, p<0.001), Asian (WMD: 20.6 ng/ml, 95% CI: 17.51 to 23.7, p<0.001) and females (WMD: 2.32 ng/ml, 95% CI: 1.34 to 3.30, p<0.001). Subjects with aa genotype have higher serum 25-OH vitamin D after administration of vitamin D supplementation compare with AA and Aa genotype (Figure 5).

Sensitivity analysis

We tested the stability of overall results by omitting one study each time by the Metan-Inf test. The results of this test show statistical stability in combined effect sizes of vitamin D supplementation on 25-OH vitamin D, TC, LDL-C, TG, HDL-C, MDA and TNF-α under the recessive model of 4 SNPs of the VDR gene.

Risk of bias assessment

Current quality assessment of included studies according to the Cochrane Collaboration Risk of Bias tool. Among 16 included RCTs, the quality of 7 and 9 studies was fair and good, respectively (ESM 2, Table 1).

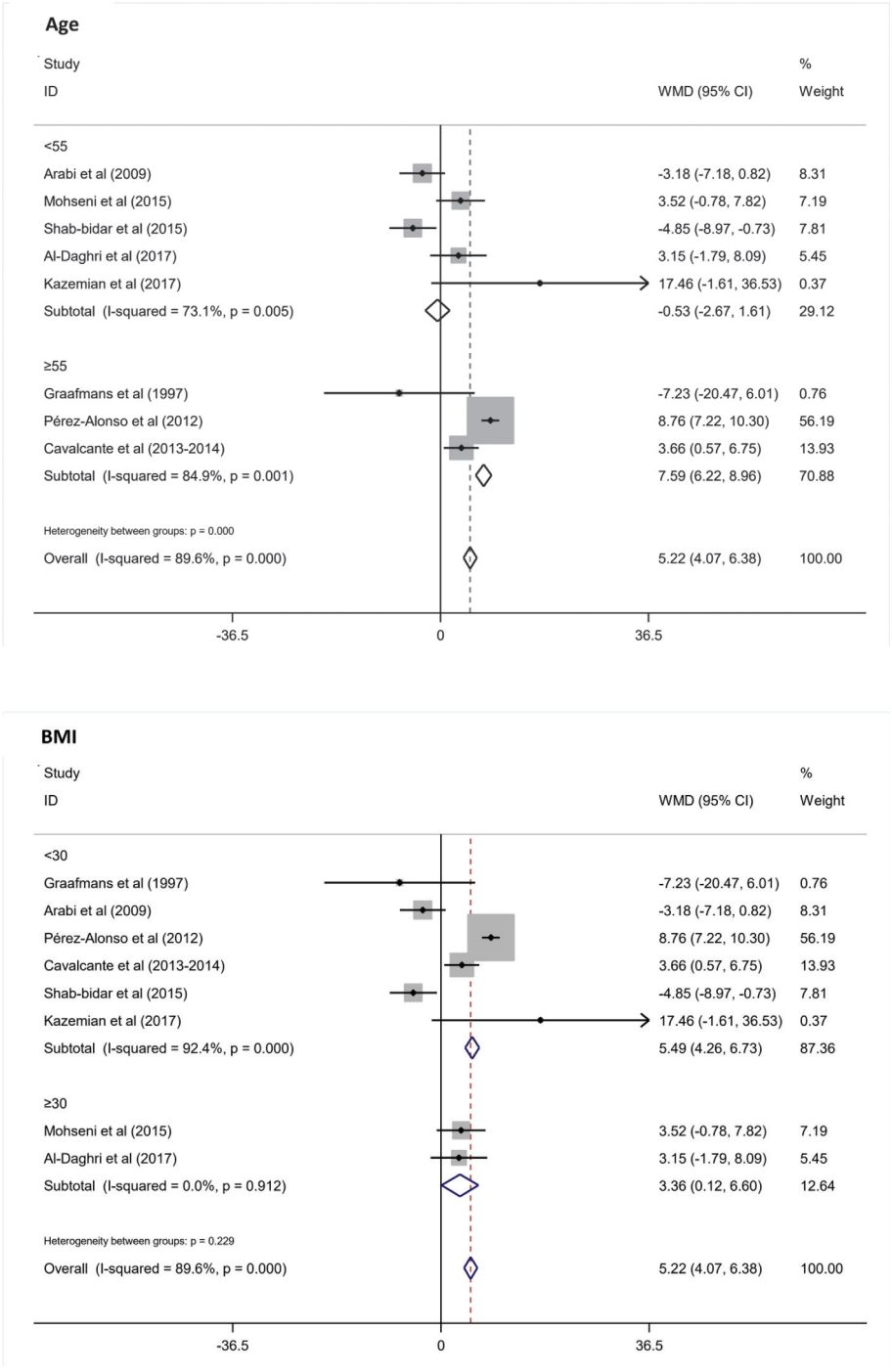
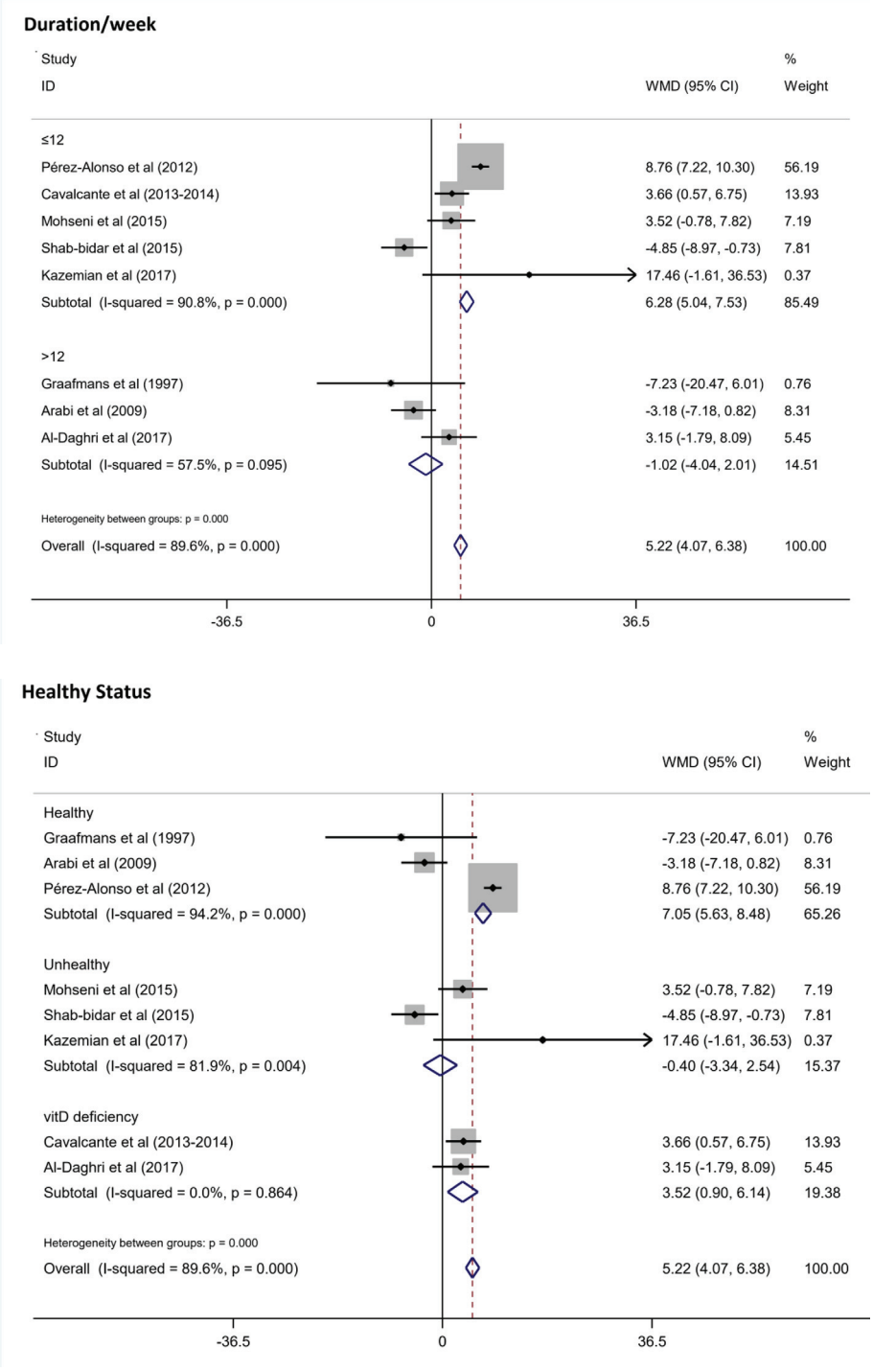


Figure 3. Subgroup analyses of vitamin D supplementation on serum concentrations of 25-OH vitamin D among BsmI polymorphism (BB, Bb) vs bb.

Publication biases

Funnels plots, Begg's and Egger's test were used to evaluate publication biases. Additionally, Begg's and Egger's test did not show any significant publication bias in all analysis (dominant contrast of pooled analysis: p=0.909 and p=0.624 for ApaI; p=0.066 and p=0.999 for BsmI; p=0.441 and p=0.652 for FokI; p=0.230 and p=0.851 for TaqI). The results showed no publication bias for results to VDR gene variants in the included studies.



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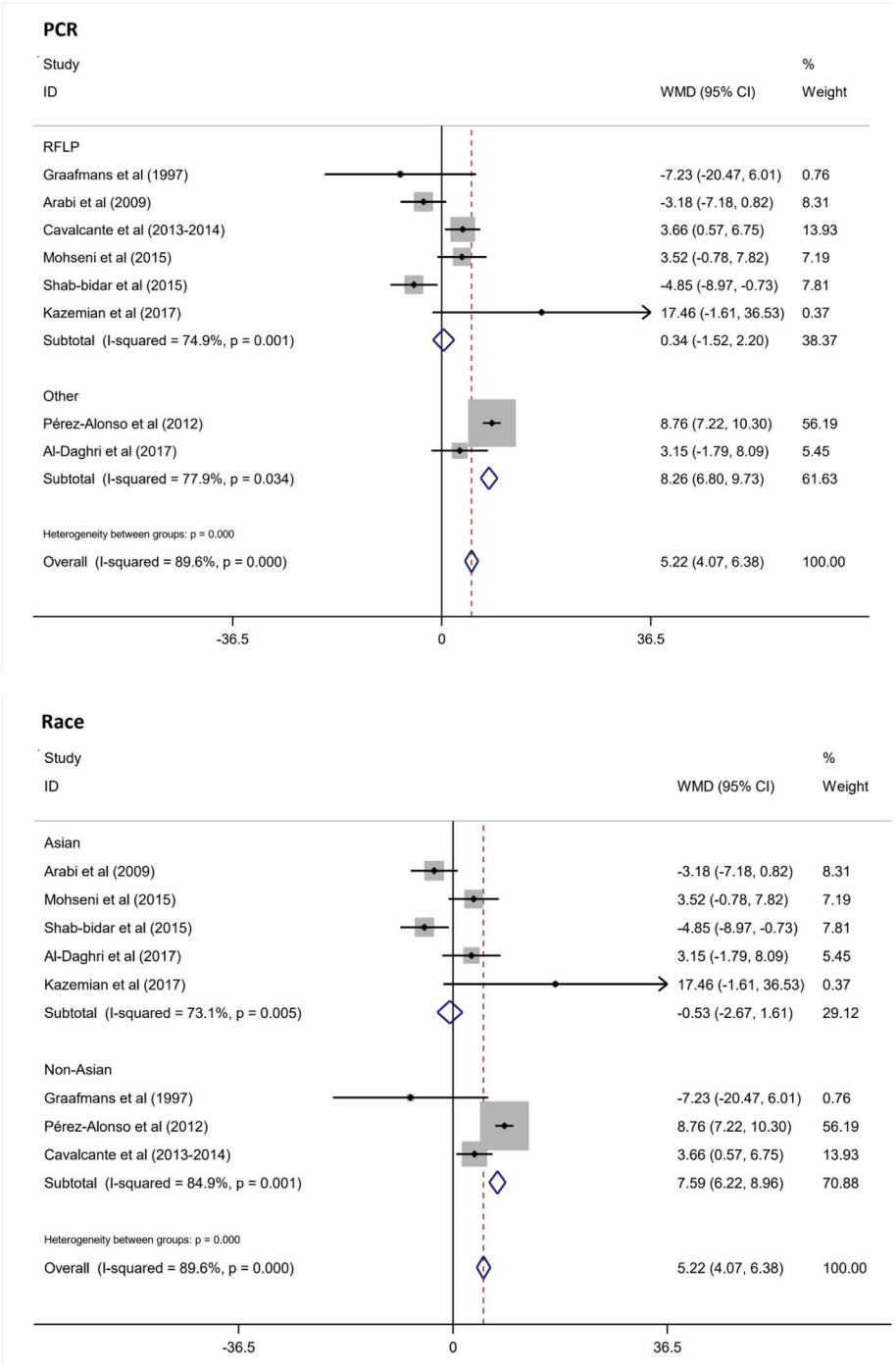
Dose-response meta-analysis

Dose-response analysis shows a considerable relationship between a high dosage (30,000–50,000 IU/Weeks) of vitamin D supplementation on increasing serum 25-OH vitamin D concentrations in BsmI, ApaI and FokI polymorphism of the VDR gene (Figure 6).

Discussion

Interaction between 4 SNP VDR (FokI, BsmI, TaqI and ApaI) and vitamin D supplementation on serum 25-OH vitamin D levels has not been significant, so this meta-analysis shows that the effect of vitamin D supplement on



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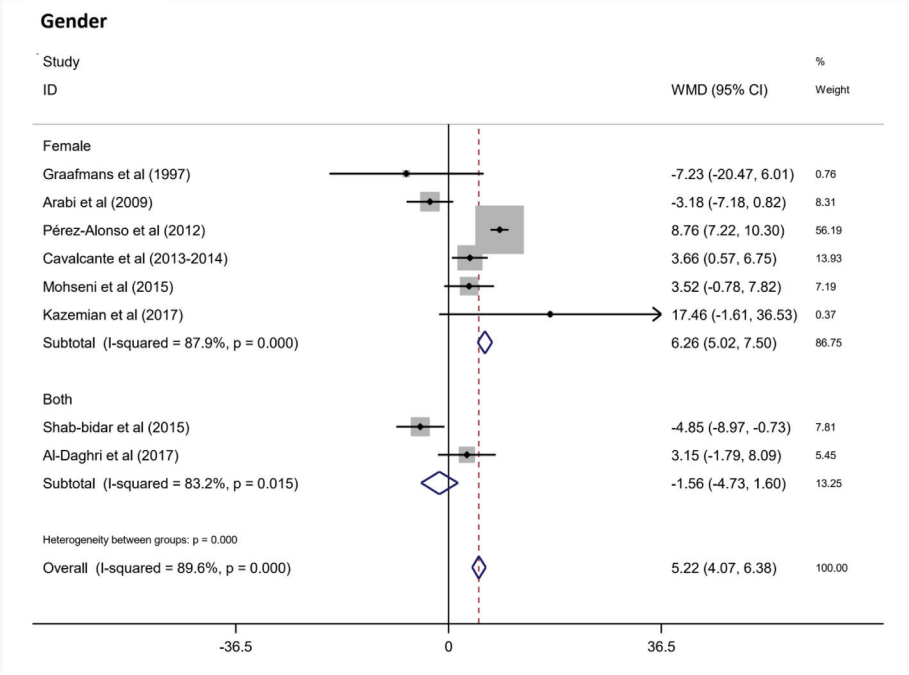
serum 25-OH vitamin D is not related to the different variations of VDR SNP. Therefore, this hypothesis that differences in serum level of 25-OH vitamin D in responses to vitamin D supplementation may be related to the different genotypes of VDR polymorphism, is very unlikely.

Further subgroup analyses for FokI, in age<years, BMI≥30, duration time ≤12 weeks, unhealthy status, HWE (N/A), RFLP-PCR, Asian, and females have shown that ff genotype has more prone to increase 25-OH vitamin D in response to vitamin D supplement compared to FF+Ff genotype. Additionally, dose-response analysis has shown



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that the dose of 30,000–50,000 IU/week can increase significantly the serum 25-OH vitamin D levels. Subjects with ff VDR genotype are expected to have more VDR activity than carriers of the FF or Ff variants that attribute a significant role to the rs2228570 f allele of the VDR gene in reducing vitamin D levels [27]. Regarding this point, the association between VDR Fok-I FF genotype and lower vitamin D status has been justifiable among these subjects. One of the reasons for this inconsistency in observations could be the interactive effects of other genes with VDR, including HLA [28]. Overall, our meta-analysis showed no significant differences in FokI genotype, although the ff variant in subgroups showed the weakest response to vitamin D supplements.

For BsmI, our subgroup analysis has displayed that BMI≥30, vitamin D deficiency and HWE (No) can be heterogeneity sources ($p>0.05$ for I^2). However, in age ≥55 years, BMI ≥30, duration time ≤12 weeks, healthy and vitamin D deficiency status, HWE (No), Other-PCR, Non-Asian and females have shown that bb genotype has more prone to increase 25-OH vitamin D in response to vitamin D supplement compared to BB+Bb genotype. Moreover, dose-response analysis has shown that the dose of 30,000–50,000 IU/weeks can increase significantly the serum 25-OH vitamin D levels, while the dose of more than 50,000 IU/weeks can be significantly decreasing the serum 25-OH vitamin D levels. The Endocrine Society has suggested that supplementation doses should reach up to 50,000 IU of vitamin D2 or D3 for individuals with vitamin D deficiency, up to this dose is effective [29]. Individuals with the bb genotype have a higher rate of VDR expression compared with subjects with the BB and Bb genotypes [30]. In addition to, the poor response in BB+Bb genotype to vitamin D supplementation may in part be mediated through poorer intestinal calcium absorption. This might be due to the compensatory increased formation of the active vitamin D metabolite, 1,25(OH)D2 from 25(OH)D by 1-a-hydroxylase, which is also expressed in pancreatic β-cells [31]. Thus, it has been suggested that homozygous bb carriers are favored for having greater receptor availability and benefit from the elevating effects on 25-OH vitamin D levels. In the presence of the B allele, there is decreased expression of VDR, making vitamin D less available to exert its modulatory effects. According to these results, there is dose-response sensitivity to 25(OH) D which increases in the following order: bb, Bb+BB. Considering the beneficial effects of vitamin D supplementation and the influence of the BsmI polymorphism of the VDR gene on the response to this supplementation, further long-term studies of larger sample sizes distributed by genotype should be conducted to elucidate the genetic interactions and metabolic traits after vitamin D supplementation in different populations.

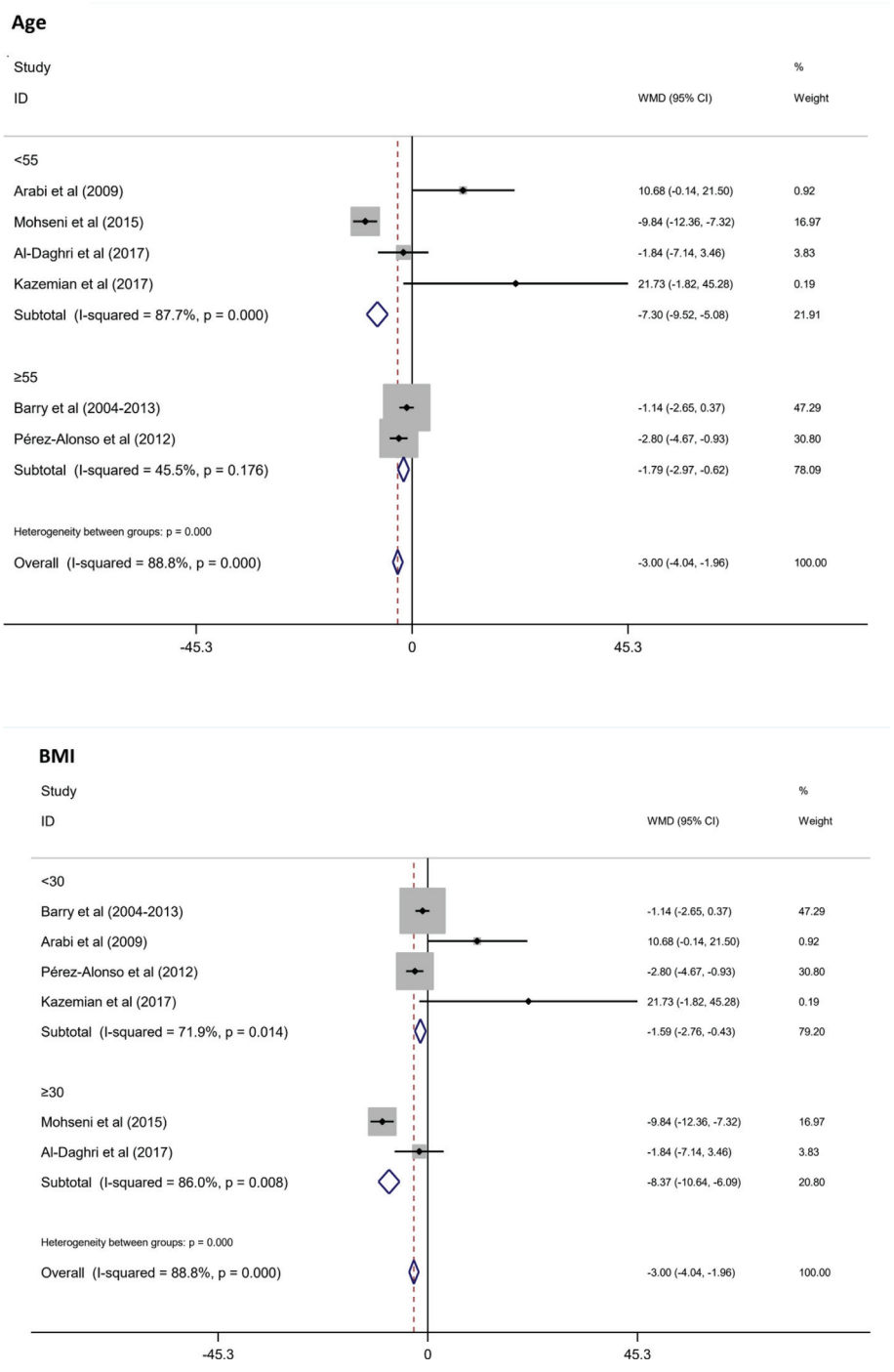
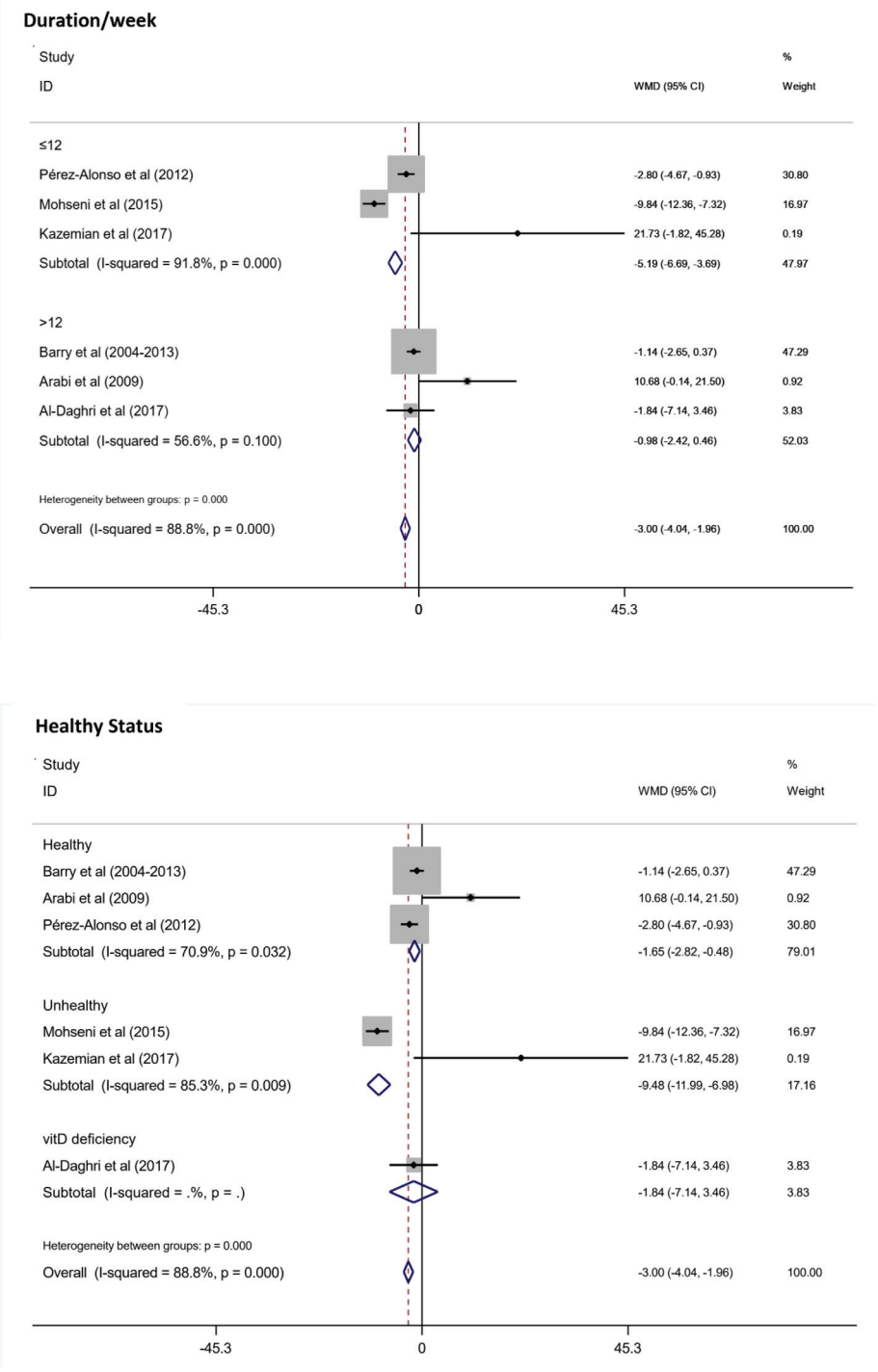


Figure 4. Subgroup analyses of vitamin D supplementation on serum concentrations of 25-OH vitamin D among TaqI polymorphism (TT, Tt) vs tt.

For TaqI, our subgroup analysis has displayed that age ≥55, duration time >12 weeks, BMI <30, non-Asian and PCR type except RFLP can be heterogeneity sources ($p>0.05$ for I^2). However, in age <55 years, BMI ≥30, duration time ≤12 weeks, unhealthy status, HWE (N/A), RFLP-PCR and Asian studies have shown that tt genotype has more prone to decrease 25-OH vitamin D in response to vitamin D supplement compared to TT+Tt genotype, But, in females have more prone to increase. We showed that individuals with the VDR TaqI tt genotypes



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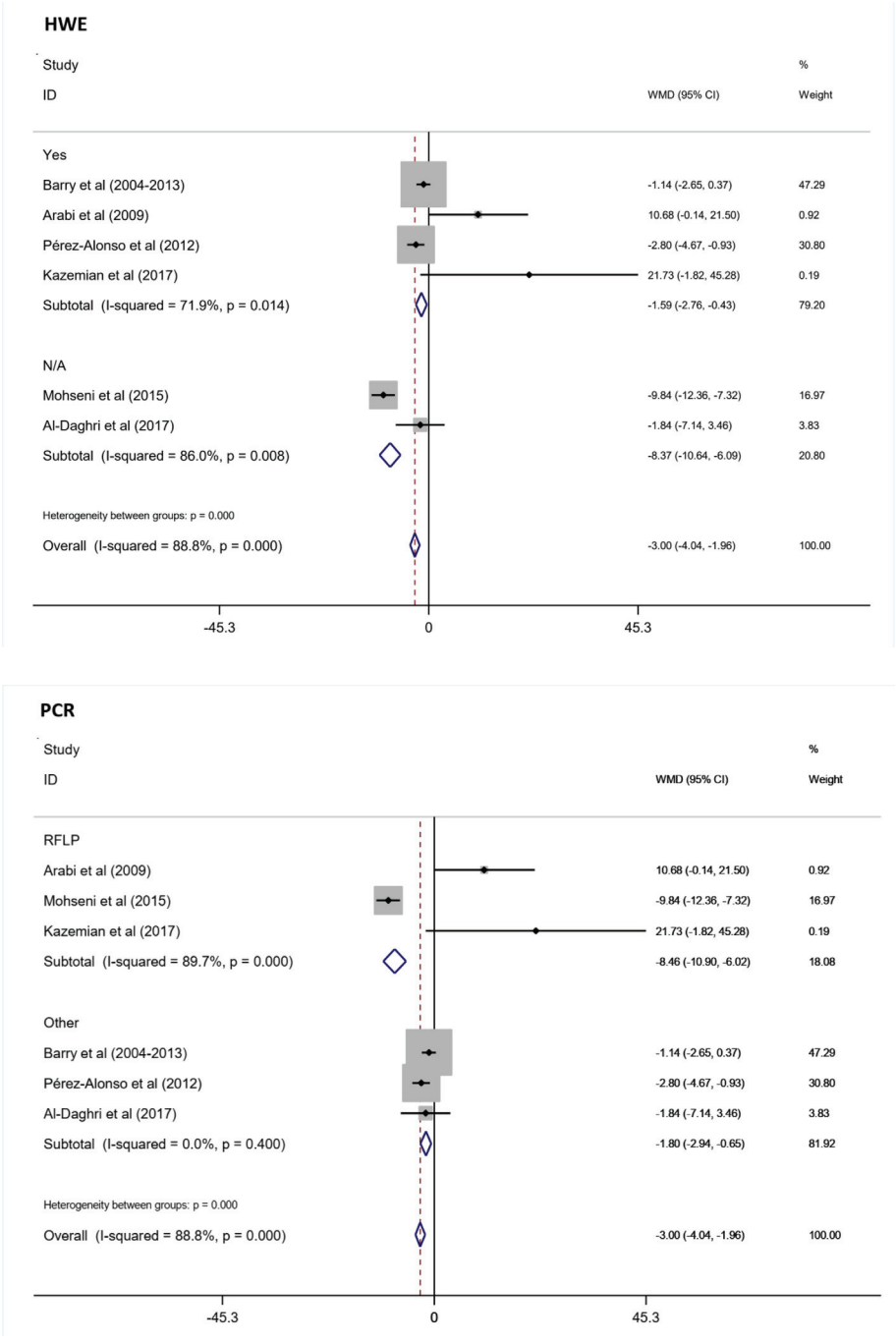
had significantly lower 25-OH vitamin D in responses to vitamin D supplementation in several subgroups. Several studies showed that subjects with the TT genotype were higher 25-OH vitamin D levels than the tt/Tt genotype [32].

For ApaI, our subgroup analysis has displayed that age ≥55, BMI <30, duration time >12 weeks, healthy status, HWE (yes), non-Asian and PCR type (other) can be heterogeneity sources ($p>0.05$ for I^2). However, in age <55 years,



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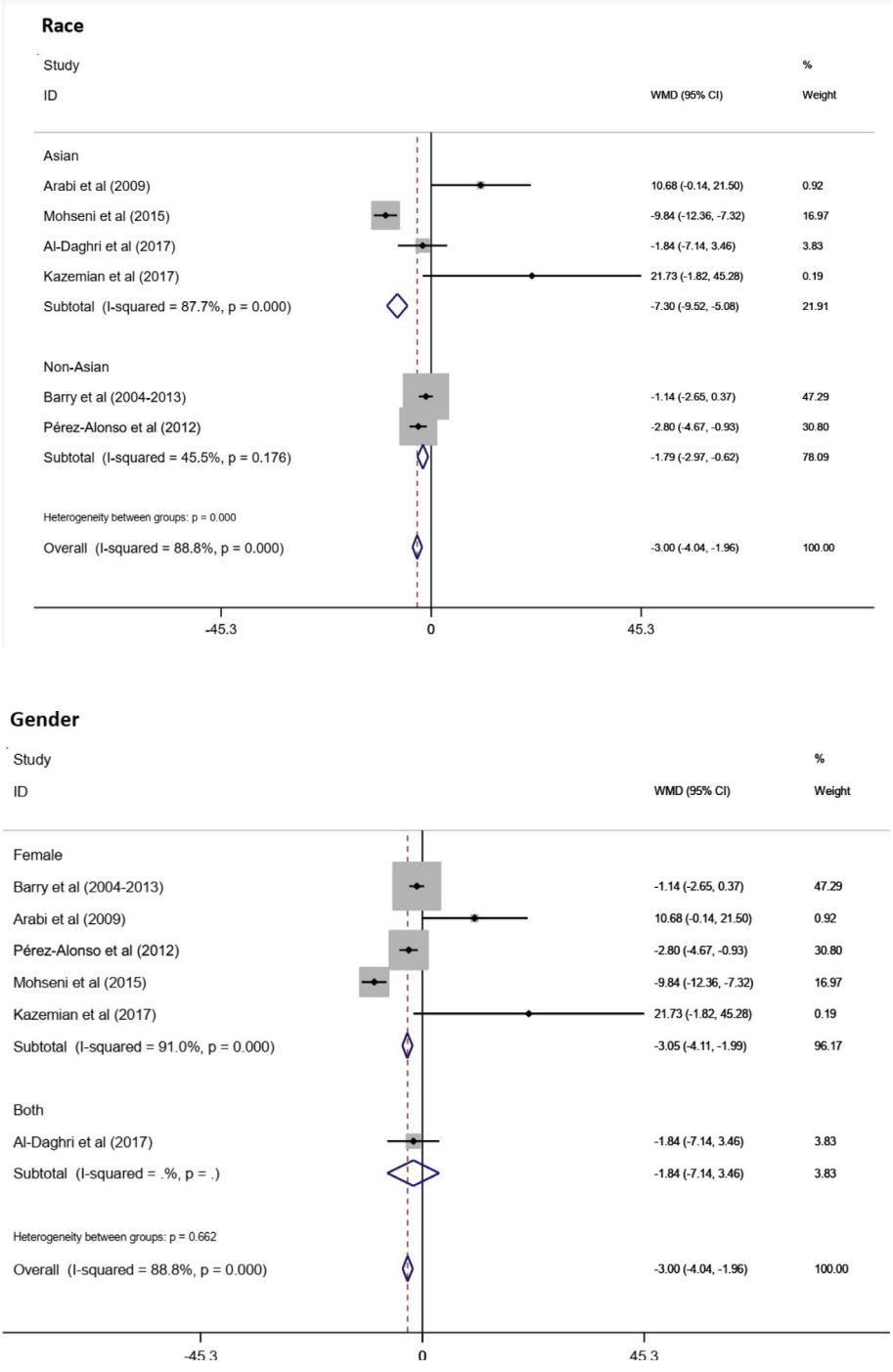
BMI ≥30, duration time ≤12 weeks, unhealthy status, HWE (N/A), RFLP-PCR, Asian and females have shown that aa genotype has more prone to increase 25-OH vitamin D in response to vitamin D supplement compared to AA+Aa genotype. Dose-response analysis has shown that the dose of less than 30,000 IU/weeks can decrease significantly the serum 25-OH vitamin D levels, while the dose of 30,000–50,000 IU/weeks can significantly increase the serum 25-OH vitamin D levels.

Interaction between FokI SNP and vitamin D supplementation on BMI, LDL and TC has not been significant, but we found a significant increment of 0.15 mmol/l, and 0.16 mmol/l in serum HDL and TG, respectively in ff genotype compared to FF+Ff genotype.



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Additionally, the interaction between BsmI SNP and vitamin D supplementation on BMI, MDA and TNF-α has not been significant. Several studies show that carriers of the ff genotype of the FokI polymorphism have lower levels of TG and HDL-C compared to two other genotypes of the FokI [33]. Vitamin D induced suppression of PTH secretion, and it has been reported that PTH could reduce lipolysis [34].

There are some limitations to the present study. Firstly, we observed some evidence of heterogeneity in included RCT. However, the heterogeneity in the combined effect sizes was disappear via the subgroup analysis. Second, it

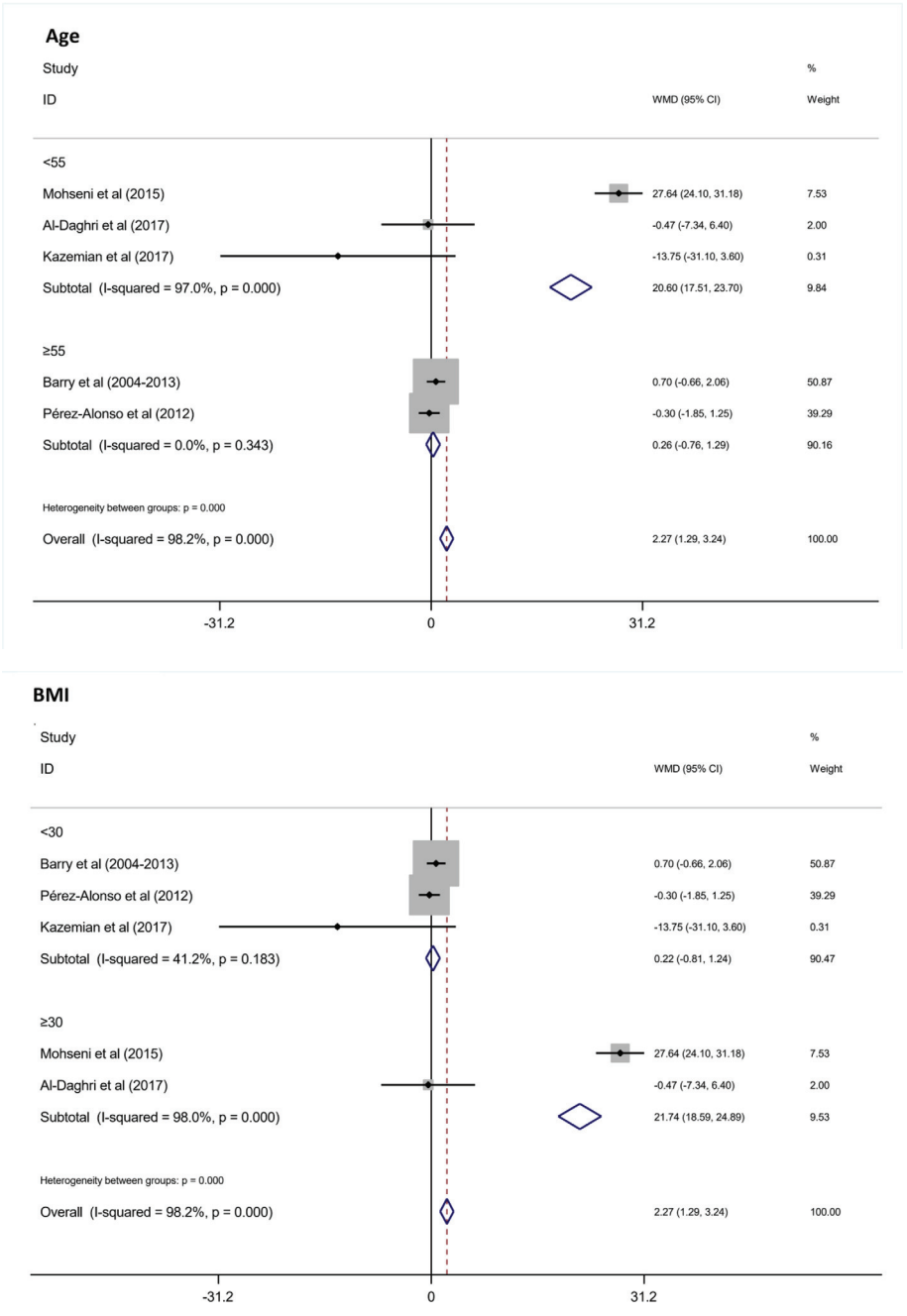
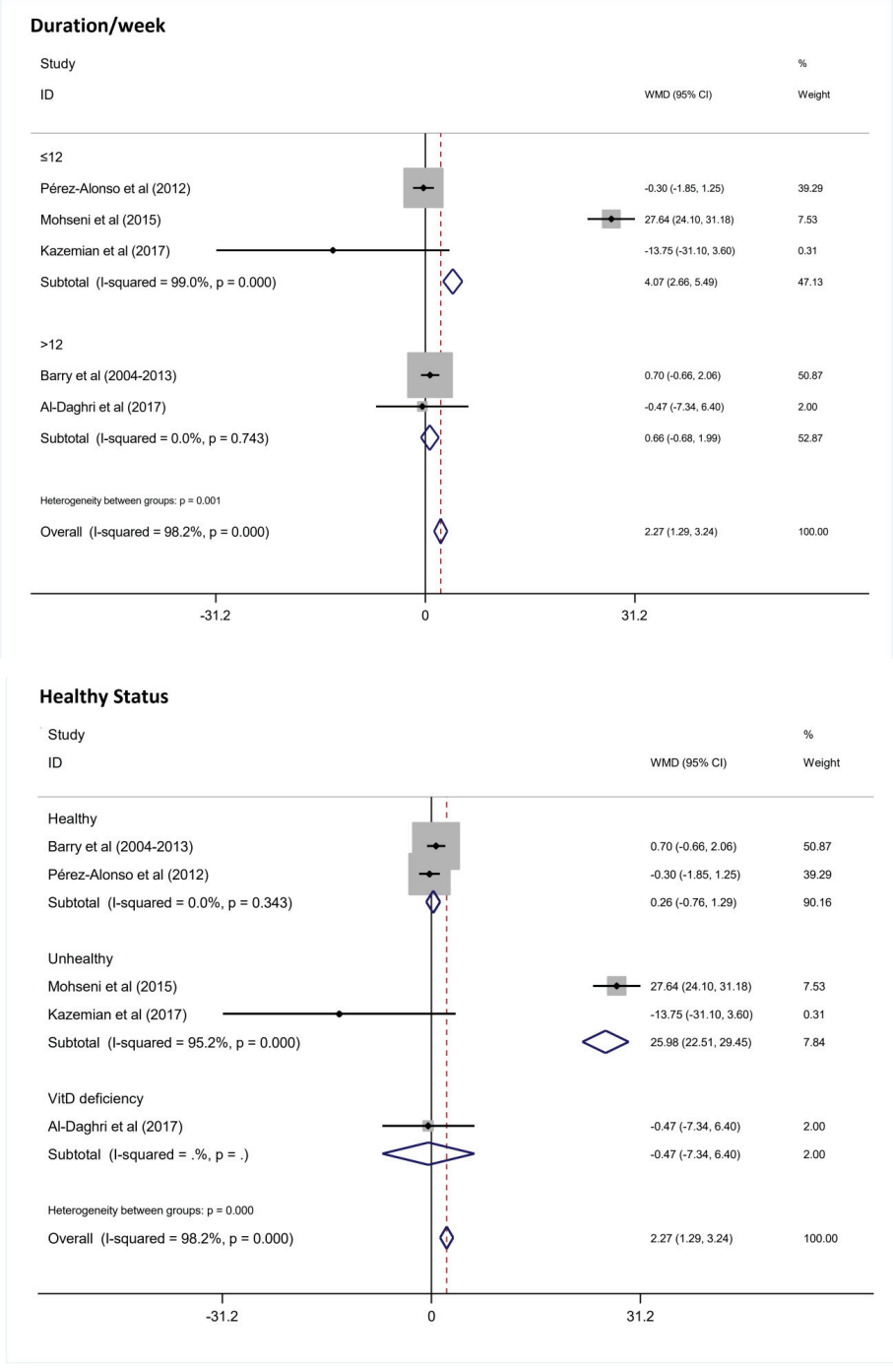


Figure 5. Subgroup analyses of vitamin D supplementation on serum concentrations of 25-OH vitamin D among ApaI polymorphism (AA, Aa) vs aa.

investigates how genetic diversity affects outcomes. Since there are ethnic variations in VDR polymorphism, these findings from genetic studies – which are connected to skin type and vitamin D synthesis – might interact with one another. Finally, up to now a small number of studies have examined these hypothesized gene-environment interactions. Despite these limitations, this study is the first and novel meta-analysis, investigating the interaction between vitamin D receptor genetic variation and metabolic outcome and serum level of vitamin D after intake vitamin D supplementation. Moreover, the quality of clinical trial studies in the present meta-analysis was good and confident.



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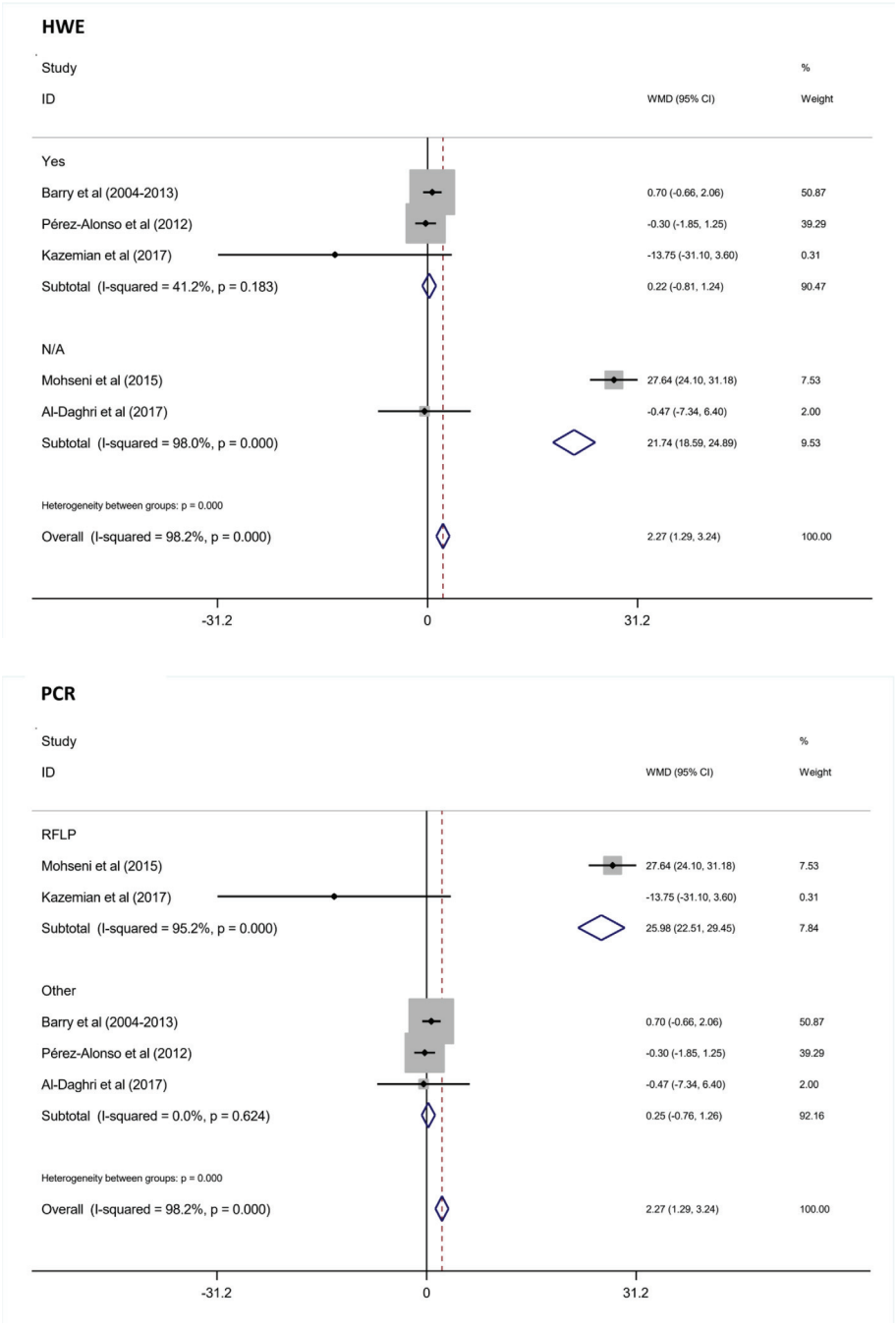
In conclusion, this meta-analysis demonstrates that the overall effect of vitamin D supplementation on serum 25-hydroxy vitamin D is independent of genetic variants of BsmI, ApaI, TaqI and FokI polymorphisms in the VDR gene, however, the elevation and reduction of 25-OH vitamin D concentrations with vitamin D supplementation might be effective in the subgroups of subjects with FokI ff, BsmI bb, ApaI aa and TaqI tt, respectively in our



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population. Overall, the results of clinical trial studies show little consistency. Methodological differences in their design, conduct and analysis may account for the discrepant findings. Therefore, additional studies should be conducted to better assess these inflammatory markers and genotypic profiles in a broader sample. Importantly, researchers should try to find fundamental molecular mechanisms of the interactions found between the above-mentioned polymorphisms with vitamin D supplementation in the future.

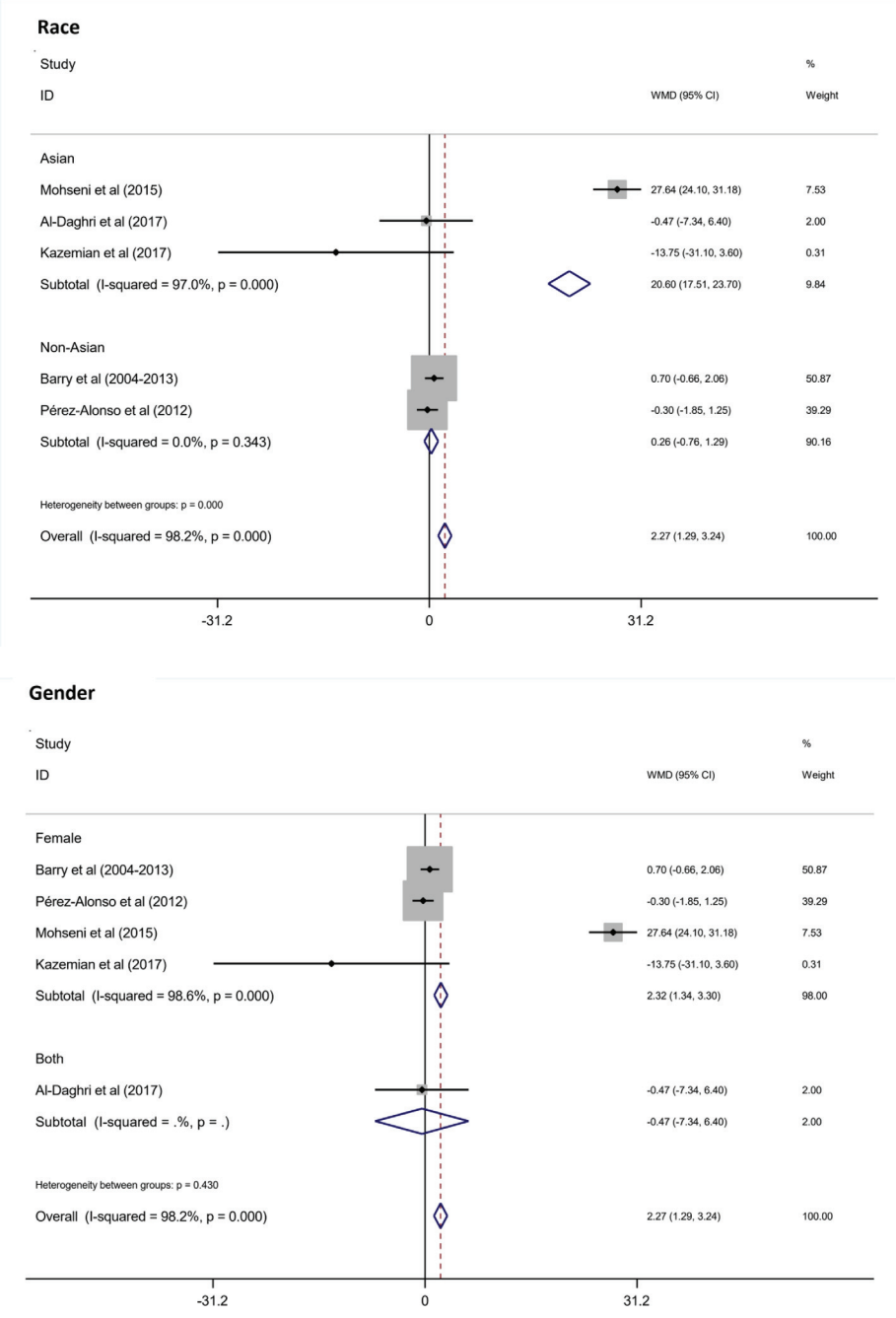

Figure 5. Continued.

Electronic supplementary material

The electronic supplementary material (ESM) is available with the online version of the article at https://doi.org/10.1024/0300-9831/a000762

ESM 1. Interaction between SNPs VDR (BsmI, ApaI, TaqI, FokI) and vitamin D supplementation on serum vitamin D and metabolic traits (Figures).

ESM 2. Risk of bias assessment for included randomized controlled clinical trials (Table).

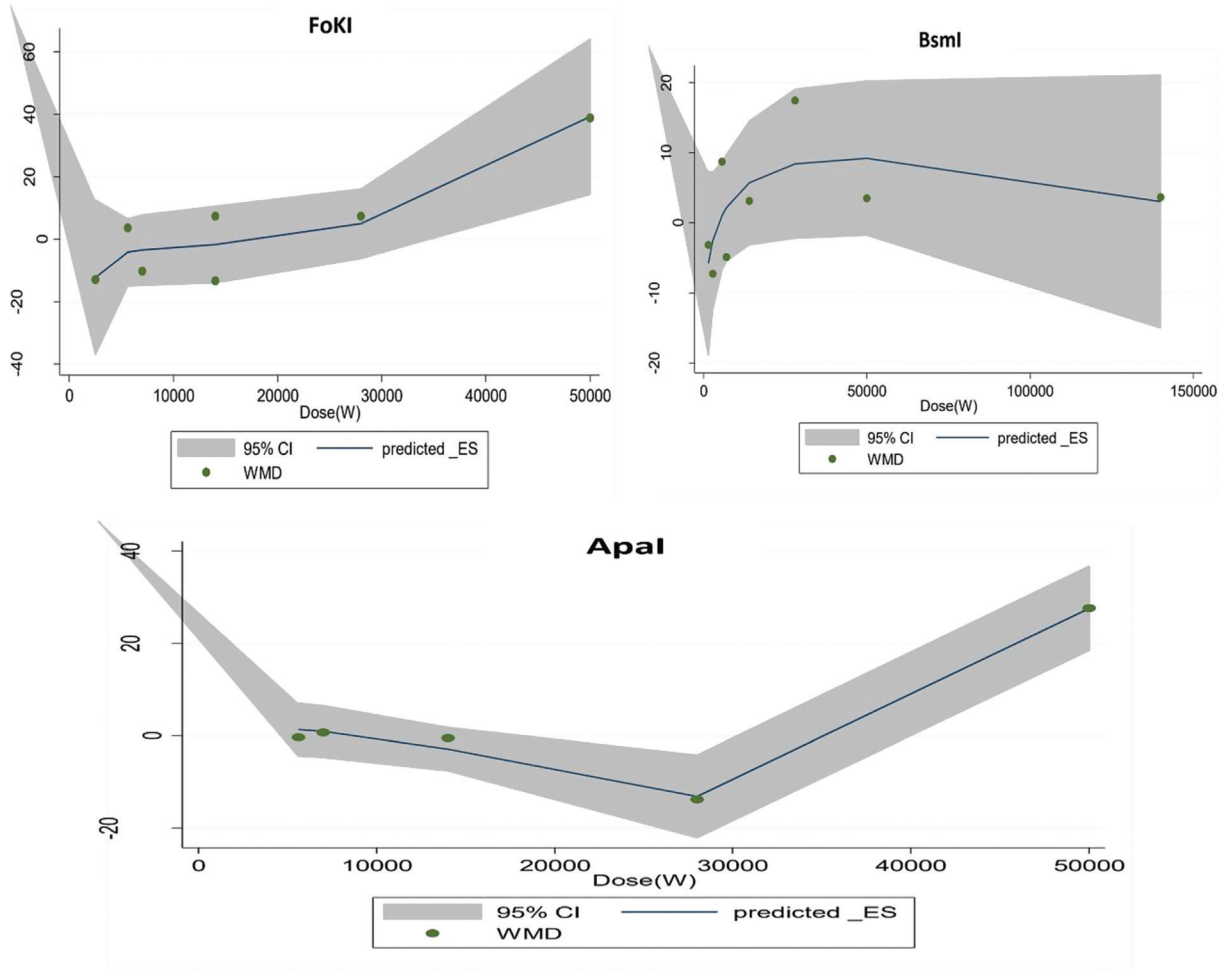

Figure 6. Non-linear dose-response of the association between dosage of vitamin D supplementation and weighted mean difference of serum concentrations of 25-OH vitamin D in BsmI, ApaI and FokI polymorphism of vitamin D receptor (VDR) gene.

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Conflict of interest
The authors declare that there are no conflicts of interest.

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